CALIBRATION OF AN INFRARED SPECTROMETER AND INFRARED ABSORPTION STUDIES OF OXIDIZED AND UNOXIDIZED LINOLEIC ACID

by

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INTRODUCTION

The work described in this paper is a part of an overall joint research project being undertaken by the physics and chemistry departments in regard to the oxidation of long chain hydrocarbons and their derivatives.

The hydrocarbon to be studied is linoleic acid, a fatty acid found in paints, varnishes, etc. Upon the oxidation of linoleic acid many changes take place such as the appearance of hydroxyl, oxy, and carboxyl groups. Little is known about these changes or the initial and secondary positions of attack, positions of double bonds, etc.

A first object of the joint project will be an attempt to determine, by use of infrared analysis, the changes which may occur upon the oxidation of the linoleic acid molecule.

A second object of the research will be an attempt to establish an infrared technique for the determination of the oxidation process which takes place. If such a technique can be perfected for the solution of the specific problem of the oxidation mechanism of the unsaturated acids, it should be equally applicable to the similar problem for gasolenes, drying oils, and other hydrocarbons. Experimentally established mechanisms of oxidation of organic compounds would be of great value in the application of organic chemicals to the fields of paints, varnishes, lubricants, fuels, plastics, etc.

The work to be described in this paper is primarily

concerned with the first object. Linoleic acid has the formula $CH_3 - (CH_2)_3-CH_2-CH=CH-CH_2-CH=CH-CH_2-(CH_2)_6-COOH$, and when linoleic acid undergoes oxidation with molecular oxygen, the peroxide formed may be cyclic (1) or open chain[1] (2,3). Knowing which of these arrangements takes place would be valuable information that could lead to a better understanding of the oxidation process.

It will be the purpose of this paper to show that the experimental results obtained indicate that the open chain arrangement is the one which occurs.

THEORY OF INFRARED SPECTROSCOPY (4,5,6)

For a clear understanding of infrared absorption spectra and their usefulness for analysis, it is necessary to recall that infrared radiation is associated with vibrations which are electromagnetic in nature. That is to say, infrared radiation is a part of the electromagnetic spectrum which goes from radiation of short wave lengths such as gamma rays, and some kinds of cosmic rays, through X-rays, ultra violet, visible and infrared, to radio waves, and beyond to slowly varying fields of longer wave lengths. The visible part of the electromagnetic spectrum has the smallest wave length interval extending from 0.4 to 0.75 microns, while the infrared region, on the other hand, includes wave lengths from

[1]Material by Dr. F. A. Kummerow in press.

0.75 to 100 microns. Because of this wider range, more information can be obtained from the infrared region than from the narrow, limited, visible range of the human eye. This wide range plus the fact that almost all organic substances have selective absorption at certain wave lengths in the infrared portion of the electromagnetic spectrum are the underlying bases of infrared spectroscopy.

The atoms of these organic molecules are assumed to be oscillating about their positions of equilibrium with very high frequencies (10^{13} - 10^{14} cycles per second). These frequencies are of the same order of magnitude as those of infrared radiation, and consequently, the molecular vibrations absorb, by resonance, all or a part of the incident infrared radiation if the frequencies of the radiation coincide with the frequencies of the molecular vibration.

Although these vibrations may appear to be quite complex, actually each is a summation of a number of simple vibrations. These simple vibrations are called normal vibrations, and are defined as those types of vibrations in which the atoms of a molecule are vibrating in phase with the same frequency, moving such that the center of gravity of the molecule does not change relative to itself. Except for cases of accidental degeneracy each normal vibration is independent of the others and can be present without affecting the other types of vibrations. Therefore, it is possible for all these vibrations to be present at one time, and yet for each one to retain its own identity, thus greatly facilitating the interpretation

of data.

Absorption of the radiation may not be due entirely to the normal vibrations of the molecule. For example, in the far infrared, the rotation of the molecule as a whole will result in some absorption. Moreover, throughout the entire infrared region, absorptions frequently occur at integral multiples (overtone bands) of the fundamental frequencies, or, at frequencies which are equal to the sum or difference (combination bands) of fundamentals. These overtone and combination bands generally absorb less than do the fundamental bands and consequently must be studied using thicker samples.

It has been experimentally observed that the frequencies of different groups of atoms are similar even though the groups are in different molecules. For example, all molecules containing the $\equiv$C-H bond have normal frequencies of about 3300 and 700cm^{-1}. An explanation of the occurence of group frequencies can easily be given in the case of the C-H, O-H, or N-H vibrations. In these cases, since the hydrogen atom is so much smaller than the other atoms, the amplitudes of the hydrogen atoms will be very much larger than the amplitudes of the other atoms. Consequently, to a first approximation, the hydrogen atom may be considered to be oscillating against an infinitely larger mass. Thus, the vibration frequency depends almost entirely on the force by which the hydrogen atom is bound to the rest of the molecule, and will be nearly the same for different molecules with the

same force constants. Experiments have also indicated that even though the bonds involve two heavier atoms, neither of which is an end atom, there will be in many cases characteristic frequencies. These characteristic frequencies have been explained on the assumption that the force constants of bonds are different when the masses are of similar magnitude. Thus, because of the different force constants involved, the propagation of the vibration of a particular bond will be damped and not transmitted appreciably to other bond groups in the molecule. On the other hand, there will again be characteristic group frequencies in a chain-like molecule, if the force constants are of similar magnitude but the masses quite different, as long as the characteristic frequencies are far apart.

Therefore, it may be stated that whenever a particular bond has a frequency that differs sufficiently from any vibrating frequency of the rest of the molecule, then this frequency will occur only slightly changed in the whole molecule and will correspond to vibrational motions in that group only. This fact, of course, is of considerable assistance in interpreting infrared data.

Measuring the frequencies of infrared radiation absorbed by a substance determines the characteristic frequencies of its molecules. A plot of the absorption values versus the corresponding frequencies gives an infrared spectrum which is characteristic of the substance being tested. Since absorption bands bear a direct relationship to the motion of

the atoms, binding forces, molecular configuration and atomic masses, the importance of such curves is quite obvious for the study of the above structural and dynamic features of the molecule, and for identification purposes.

DESCRIPTION OF THE SPECTROMETER

Source of Radiation

The instrument used was a Perkin-Elmer spectrometer, model 12A, PLATE I. An electrically heated, water cooled carborundum rod serves as the source of infrared radiation. It may be heated up to a temperature of 1000°centigrade by passing through it a current of 5-6 amperes at 40-50 volts. The power supply to the carborundum rod consists of a constant voltage transformer together with a variac for adjustment.

Optical Path and Dispersing Medium

The optical path of the instrument is shown in the accompanying PLATE II. The radiation beam from the source is focused through the sample cell, C, onto the entrance slit, S_1; then collimated by the off-axis parabolic mirror, M_{111}; dispersed by the prism, P; reflected by the Littrow mirror, M_{1111}; again dispersed by the prism and eventually brought to a focus on the exit slit, S_{11}. The exit slit passes only rays of a narrow frequency band, after which the rays are focused onto a compensated vacuum thermocouple maintained at high vacuum by a carbon getter bulb. Since aluminum surfaces are quite permanent and

are highly reflective in the infrared, front surface aluminized mirrors are used for all the optical parts except the prism and the housing windows. The windows and the prism are made of rock salt because wave lengths transmitted by rock salt (2.5 - 15 microns) include most of the absorption bands which may be assigned to characteristic groups in the molecule. The two slits are 12 mm high and are jointly controlled by a slit micrometer, M_s, reading in thousandths of a mm from 0 to 2 mm. Wave length selection is obtained by the rotation of the Littrow mirror M_{1111} which is controlled by the wave-length micrometer, M_w. The calibration of the drum of the wave-length micrometer is arbitrary and reads directly in hundredths from 0 to 20.

Infrared Detector and Amplifier

Since infrared radiation cannot be photographed, and since there are no photo-electric elements which are sensitive to infrared radiation, the detecting device must necessarily be of a thermosensitive nature such as a bolometer, thermocouple, etc. The device used with this instrument is a high vacuum, compensated type thermocouple with the following properties.

Junction ..... bismuth-bismuth tin.
Total resistance ..... 12 ohms.
Area of target ..... 0.6 x 4 mm.
Window ..... Potassium Bromide.
Approximate response time ..... 1 sec.
Sensitivity .....0.3 microvolt/erg/sec.

The output of the thermocouple is indicated by a sensitive galvanometer (L and N galvanometer #22840) mounted in a Mueller suspension mounting (7) to eliminate vibrational disturbances. The rotation of the galvanometer mirror is magnified by aiming a spot light on the mirror and allowing the reflected light to be focused onto the spectrometer scale at a distance of about seven meters from the mirror. Thus, a small rotation of the mirror will result in a correspondingly large deflection reading on the spectrometer scale, thereby, increasing the sensitivity of the spectrometer.

The housing enclosing the thermocouple is maintained at a high vacuum by a carbon getter bulb immersed in liquid air during the operation of the spectrometer to take advantage of a more than tenfold increase in sensitivity in going from atmospheric pressure to 10^{-4} mm of mercury. Out gassing of the thermocouple housing and getter bulb is accomplished by a high vacuum, oil bath mechanical pump.

CALIBRATION OF THE SPECTROMETER

Calibration of the instrument was accomplished by obtaining the absorption spectra of several common substances whose absorption spectra are well known such as water vapor, carbon dioxide, and ammonia vapor. By comparing the obtained absorption curves with the corresponding known curves of the various substances, it was possible to assign the proper frequencies to the various absorption regions of the obtained curves, Fig. 1 and Table 1. Plotting the frequencies in cm^{-1}. against the

corresponding micrometer settings for as many absorption bands as was considered necessary gave the desired calibration curve. See Fig. 2. As a final step, the points were adjusted to give a smooth curve.

ABSORPTION CELL AND DESCRIPTION OF SAMPLES

The absorption cell consists of two rock salt plates separated by a lead foil spacer. Each of the rock salt plates is about one inch square and 3/8 inch think, while the spacer is about one inch square and .08 mm thick. New spacers were used for each sample, and the entire cell was cleaned with grain alcohol each time a new sample was introduced, Fig. 3.

Data were taken for one unoxidized linoleic acid sample, and four samples with varying degrees of oxidation. A temperature of 65 degrees centigrade for oxidation times of 20, 60, 75, and 90 minutes gave the varying degrees of oxidation. The degree of oxidation of each sample was obtained by determining its peroxide value (millimoles of oxygen per gram). These pv values were found to be 96.1, 133.7, 207.1, and 356.

EXPERIMENTAL PROCEDURE AND RESULTS

The values of percentage absorption for the five samples were obtained for frequencies ranging from 700 to 3900 cm^{-1}. To eliminate the undesirable absorption due to carbon dioxide or water in the air, a spectrometer reading was taken with the cell out of position, together with a spectrometer reading with the cell in position. The ratio of the reading with

the cell in position to the reading with the cell out of position gives the per cent transmission allowed by the sample. If the small radiation losses due to reflection are neglected, a subtraction of the per cent transmission from one hundred per cent will, of course, give the per cent absorption due to the sample. Plotting the absorption values against their corresponding frequencies gives the desired absorption curve.

Three of the five curves which were obtained are shown on PLATE III. To avoid confusion the two remaining curves have not been included on PLATE III, but are shown on PLATE IV.

DISCUSSION

During the oxidation of linoleic acid, it is believed that a heterogeneous mixture of compounds including hydroperoxides, peroxides, alcohols, ketones, aldehydes, acids, etc. are formed. Consequently, in the spectrum of oxidized and unoxidized linoleic acids some of the absorption bands should possibly be found which are due to -CH=C-, CH-CH_2, C=O (free acids), C=O (aldehydes and ketones), -C=C- (non conjugated), C-H (aliphatic), C-H (olefinic), O-H (bonded), and O-H (free). These absorption bands have been found to occur at the following frequencies.[2]

[2]Restricted material in project report of American Meat Institute Foundation.

-CH=CH-	970
-CH-CH_2	990
C-O	1200-1250
C=O (free acids)	1650-1740
C=O (aldehydes and ketones)	1675-1730
-C=C- (non-conjugated)	1600-1650
C-H (aliphatic)	2800-3000
C-H (olefinic)	2975-3075
O-H (bonded)	3100-3520
O-H (free)	3730-3520

By reference to PLATES III and IV it will be seen that absorption bands do appear in the regions listed above. A comparison of the spectrum of the unoxidized sample, PLATE III with the oxidized samples PLATES III and IV, will show that, in some cases, after oxidation the absorption has been increased, decreased, or perhaps the band has been shifted to an adjacent region.

These changes, of course, are probably due to the presence of the complex compounds, mentioned above, which may form during the oxidation process. Further investigations are planned along those lines, with the hope that the information obtained will be of great assistance in determining the complete oxidation mechanism of linoleic acid.

It is believed that the oxidation of linoleic acid may take place in either of two ways. Kummerow[3], Bolland and Gee (3) and Farmer (2) have presented chemical evidence that two oxygen atoms are added between the C and H atoms of an active methylene group to form a hydroperoxide of the form

[3]Material by Dr. F. A. Kummerow in press.

$-\overset{|}{\underset{|}{C}}-O-O\cdot H$. However, according to Engler (1), the oxidation of linoleic acid results in a cyclic arrangement of the form

$$\begin{array}{c} -\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}- \\ O-O \end{array}$$

In the first case, as oxidation is increased, an increase in C-O and O-H absorption values, and a decrease in C-H absorption should occur. In the second case, an increase in C-O absorption, but no change in O-H or C-H absorptions should occur.

Referring to PLATE V, it may be seen that as oxidation proceeded there were relatively large increases in C-O and O-H absorptions, while there was an almost negligible amount of increase in C-H absorption. The results, with the exception of the C-H absorption changes which should show a decrease rather than an increase, favor case one. Perhaps, this exception was a result of small unavoidable errors due to the inability to maintain perfect uniformity in the preparation of the samples relative to cell thickness, etc. Or, perhaps the resolving power of the spectrometer was insufficient to indicate accurately the constancy in C-H absorption that should have taken place. At any rate, the results seem to indicate that the first type of oxidation ($-\overset{|}{\underset{|}{C}}-O-O-H$) occurs, thus substantiating the work of Kummerow, Bolland, Gee, and Farmer.

In conclusion, it must be stated that the experimental results obtained in this work merely indicate, and do not prove that the open chain type of oxidation is the type which occurs. To prove this definitely will require obtaining the

spectra of substances which are known to oxidize in a cyclic manner, and also the spectra of substances which are known to have the open chain arrangement. By comparing these spectra with the spectra of oxidized and unoxidized linoleic acid, a definite conclusion concerning this problem can probably be made. Work is being planned along these lines.

EXPLANATION OF PLATE I

Fig. 1. View of the spectrometer only

A. Slit Control.

B. Micrometer screw.

C. Housing with sample cell between.

D. Spectrometer scale.

E. Water cooling system.

F. Mirrors which reflect galvanometer deflections onto the spectrometer scale.

Fig. 2. View of the rest of the apparatus.

A. Galvanometer housing.

B. Spot-light.

C. Control for adjusting galvanometer mirror.

D. Variac.

PLATE I

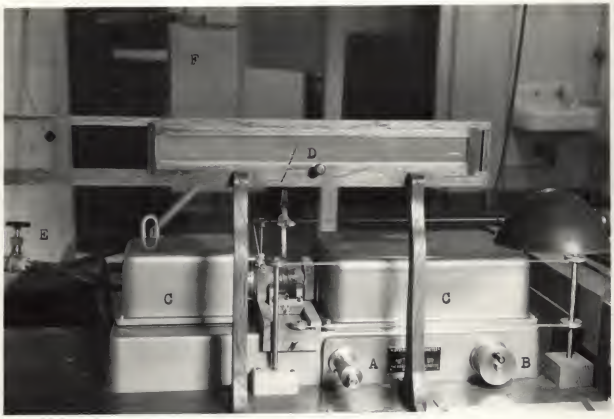


Fig. 1. View of the spectrometer only.

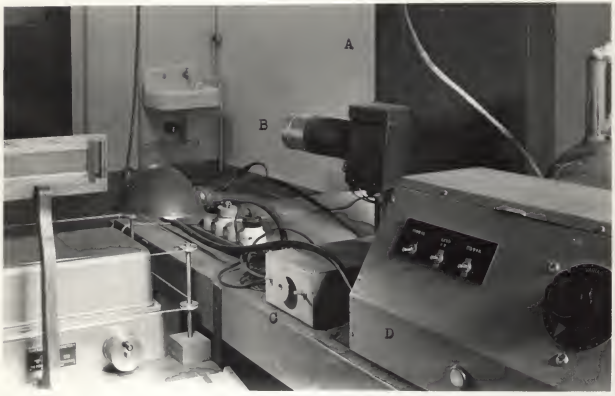
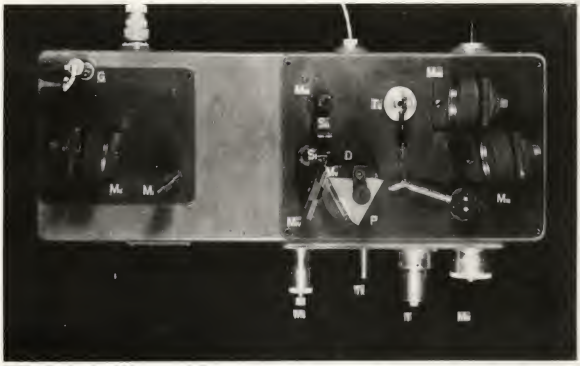


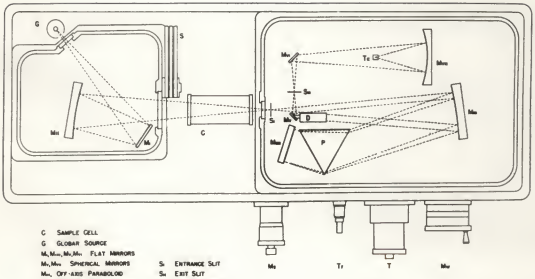
Fig. 2. View of the rest of the apparatus.

EXPLANATION OF PLATE II

In the upper part is shown a photographic plan view of the spectrometer with the housing removed. A schematic diagram of the spectrometer is shown in the lower view, indicating the optical system and the path which the radiation follows.

PLATE II





C Sample Cell
G Globar Source
M_1, M_2, M_3, M_4, M_5 Flat Mirrors
M_z, M_m Spherical Mirrors
M_m Off-axis Parabolod
M_s Slit Micrometer
M_w Wavelength Micrometer
P Prism
S Shutters
S_i Entrance Slit
S_e Exit Slit
T Turret Drum
T_r Turret Release
T_c Thermocouple
D Temperature Compensator

Perkin-Elmer Infrared Spectrometer
Model 12A

EXPLANATION OF PLATE III

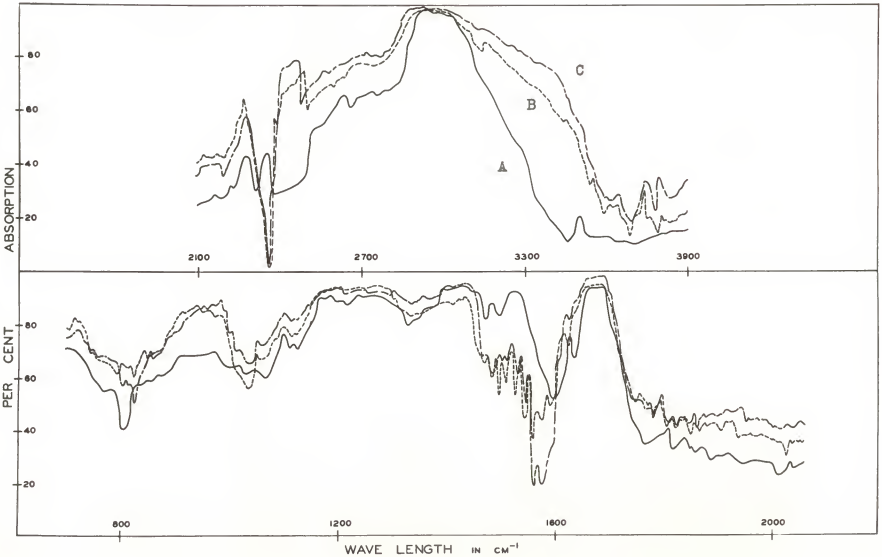
The absorption spectra of three of the samples are shown.

A. The unoxidized sample of linoleic acid.

B. Linoleic acid sample which has been oxidized for 75 minutes at 65 degrees centigrade. The pv value was 207.1.

C. Linoleic acid sample which has been oxidized for 90 minutes at 65 degrees centigrade. The pv value was 356.

PLATE III



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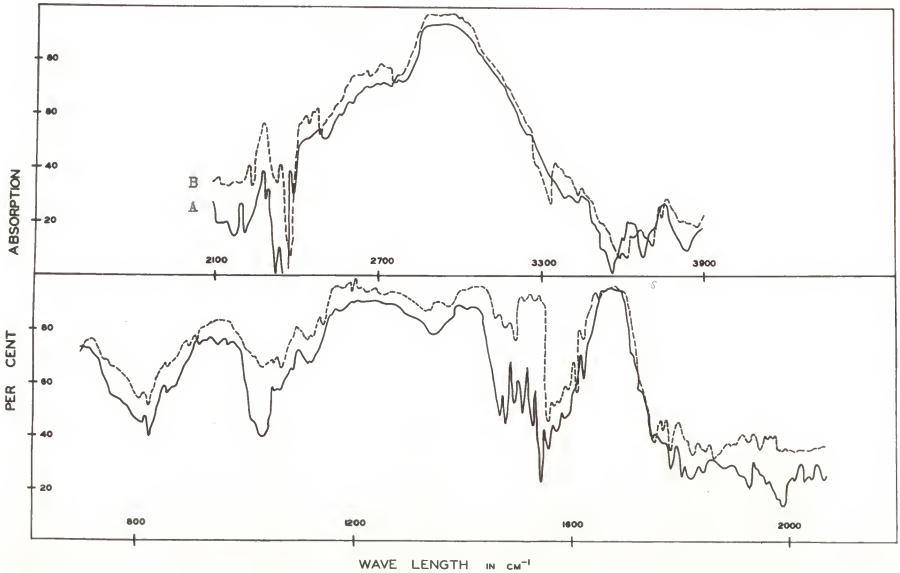
EXPLANATION OF PLATE IV

The absorption spectra of two of the oxidized samples are shown.

A. Linoleic acid sample which has been oxidized for 20 minutes at 65 degrees centigrade. The pv value was 96.1.

B. Linoleic acid sample which has been oxidized for 60 minutes at 65 degrees centigrade. The pv value was 133.7.

PLATE IV


ABSORPTION
80
60
40
20
B
A
2100
2700
3300
3900
PER CENT
80
60
40
20
800
1200
1600
2000
WAVE LENGTH IN CM⁻¹

EXPLANATION OF PLATE V

Plot of data showing the increase in per cent absorption with increase in oxidation (pv value) for various atom groups.

A. C-H bond at 2940 cm^{-1}.

B. C-O bond at 1205 cm^{-1}.

C. O-H (free) bond at 3650 cm^{-1}.

D. O-H (free) bond at 3740 cm^{-1}.

E. O-H (bonded) bond at 3460 cm^{-1}.

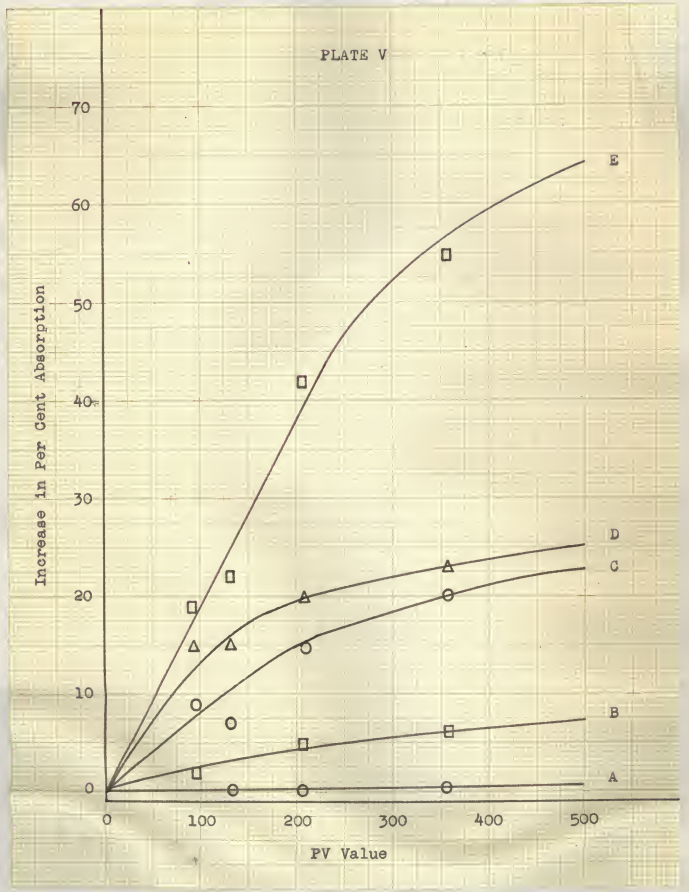


PLATE V
Increase in Per Cent Absorption
70
60
50
40
30
20
10
0
0
100
200
300
400
500
PV Value
E
D
C
B
A

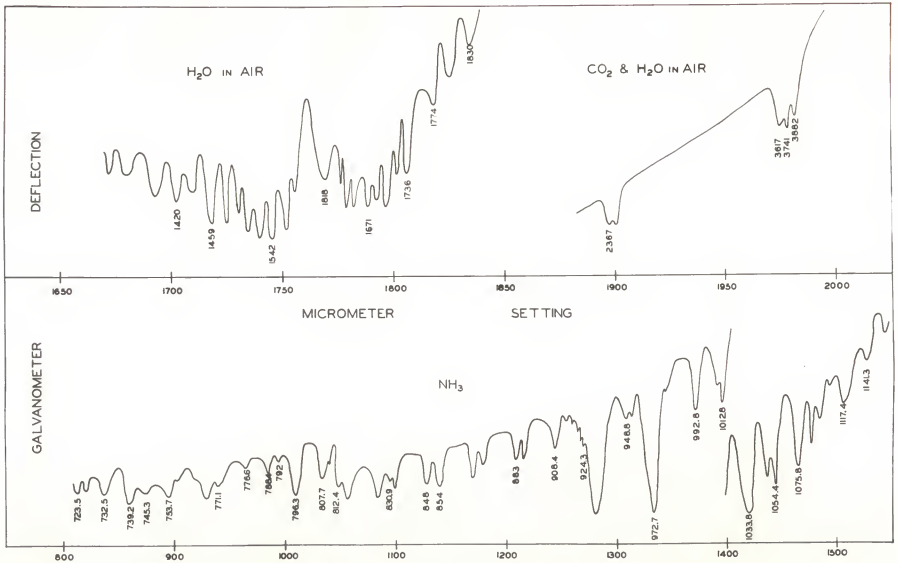


Fig. 1. Absorption spectra of NH_3, H_2O in air, and CO_2 and H_2O in air.

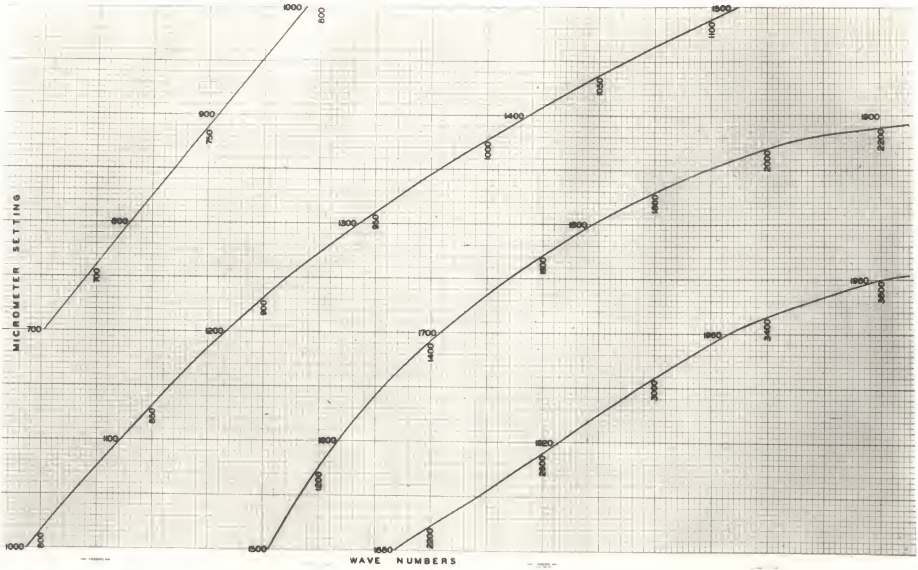
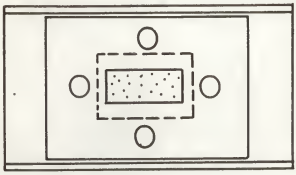
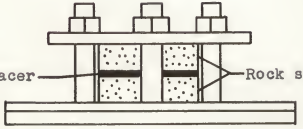


Fig. 2. Calibration curve.



Plan View with screws and nuts removed.



Side elevation.

Fig. 3. Absorption cell.

Table 1. Data for plotting calibration curve.

	Micrometer setting	Wave length in cm^{-1}
NH_3	815	723.5
	837	732.5
	860	739.2
	876	745.3
	896	753.7
	940	771.4
	964	776.6
	985	788.4
	995	792.0
	1009	796.3
	1034	807.7
	1049	812.4
	1096	830.9
	1128	848.0
	1141	854.0
	1209	883.0
	1245	908.4
	1282	924.3
	1310	948.8
	1344	972.7
	1372	992.8
	1396	1012.6
	1421	1033.8
	1444	1054.4
	1466	1075.8
	1507	1117.4
	1527	1141.3
H_2O	1703	1420.0
	1719	1459.0
	1746	1542.0
	1770	1618.0
	1790	1671.0
	1806	1736.0
	1819	1774.0
	1834	1830.0
CO_2	1898	2336.0
	1900	2367.0
	1974	3617.0
	1978	3741.0
	1981	3882.0

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Appreciation is also expressed to E. L. Brosius of the Department of Chemistry who prepared the samples of oxidized and unoxidized linoleic acid.

LITERATURE CITED

(1) Engler, C.
Concerning the so called activation of oxygen and peroxide formation. (In German). Berichte der deutscher chemischen gesellschaft. 30: 1669-1682. June 1897.

(2) Farmer, E. H.
Peroxidation in relation to olefin structure. Transactions Faraday Society. 42:228. 1946.

(3) Bolland, J. L. and Geoffry Gee.
Kinetics of oxidation of unconjugated olefins. Transactions Faraday Society. 42:236. 1946.

(4) Herzberg, Gerhard.
Infrared and raman spectra of polyatomic molecules. New York. D. Van Nostrand. 632 p. 1945.

(5) Instruction manual-infrared spectrometer model 12A. Glenbrook, Conn. Perkin Elmer Corp. 43 p. 1945.

(6) Barnes, R. R., C. Gore, U. Liddel, and V. Z. Williams. Infrared spectroscopy-industrial applications and bibliography. New York. Reinhold Publishing Co. 236 p. 1944.

(7) Strong, John.
Procedures in experimental physics. New York. Printer-Hall. 642 p. 1945.

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